

(12) UK Patent Application (19) GB (11) 2 218 514 A (13)
(43) Date of A publication 15.11.1989

(21) Application No 3906374.7

(22) Date of filing 20.03.1989

(30) Priority data

(31) 193027

(32) 12.05.1988

(33) US

(71) Applicant

General Motors Corporation

(Incorporated in the USA - Delaware)

3044 West Grand Boulevard, Detroit, Michigan 48202,
United States of America

(72) inventors

Peter Shu-Ti Lee

Richard F Majkowski

Dale L Partin

(74) Agent and/or Address for Service

A D Haines

Patent Section (F6) Vauxhall Motors Ltd, P O Box 3,
Kimpton Road, Luton, Beds, LU2 0SY,
United Kingdom

(51) INT CL

G01N 21/35 33/497

(52) UK CL (Edition J)

G1B 3BG

U1S S1032

(56) Documents cited

None

(58) Field of search

UK CL (Edition J) G1B 3AA 3BG

INT CL G01N

(54) Process evaluation by isotope enrichment

(57) An evaluation of an engineering or biological process or system can be made by treating the system with an isotopically-enriched substance which flows through the system intact or which is changed into another substance containing isotope-enrichment, and a gaseous sample thereof is analyzed at low pressure e.g. by infra-red spectroscopy to measure the intensity of a specific absorption line of the enriched isotopic species whereby the concentration of the species is calculated. The enrichment of the measured species furnishes information about the functioning of the process. In bio-medical testing, the tracer isotopic species used frequently are enriched values of CO, CO₂, H₂O or NH₃ in the breath or derived from tissues or other specimens.

GB 2 218 514 A

BEST AVAILABLE COPY

1/2

2218514

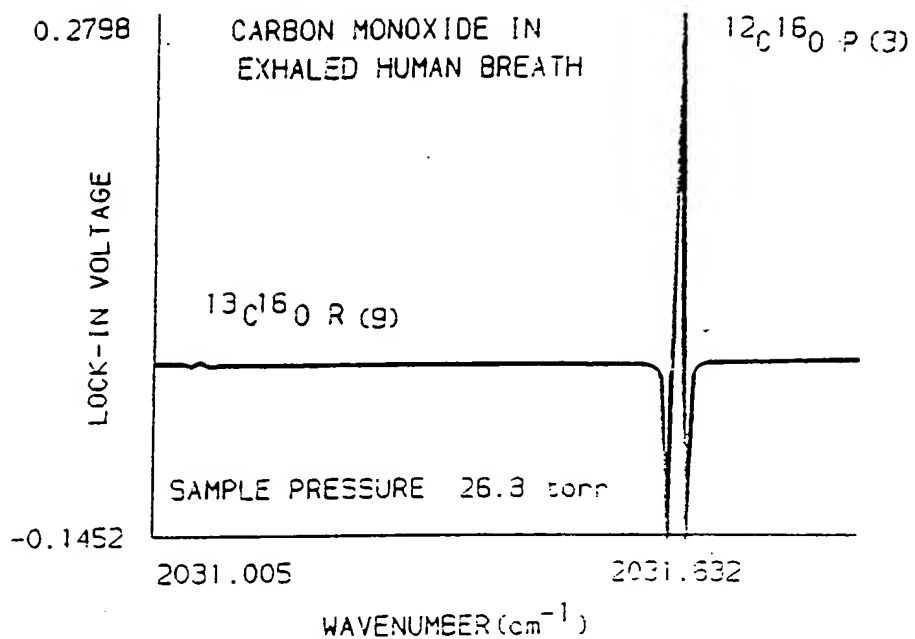


FIG. 1

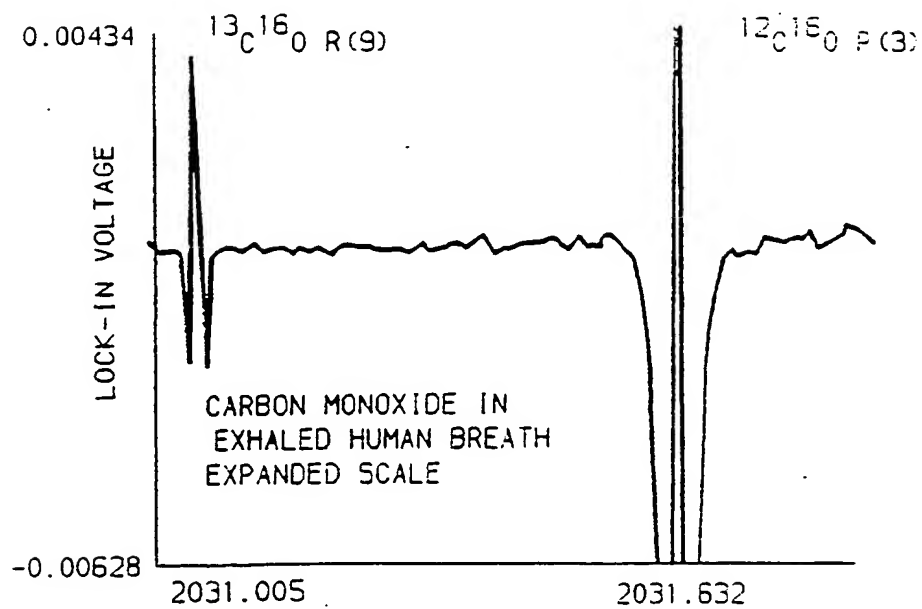


FIG. 2

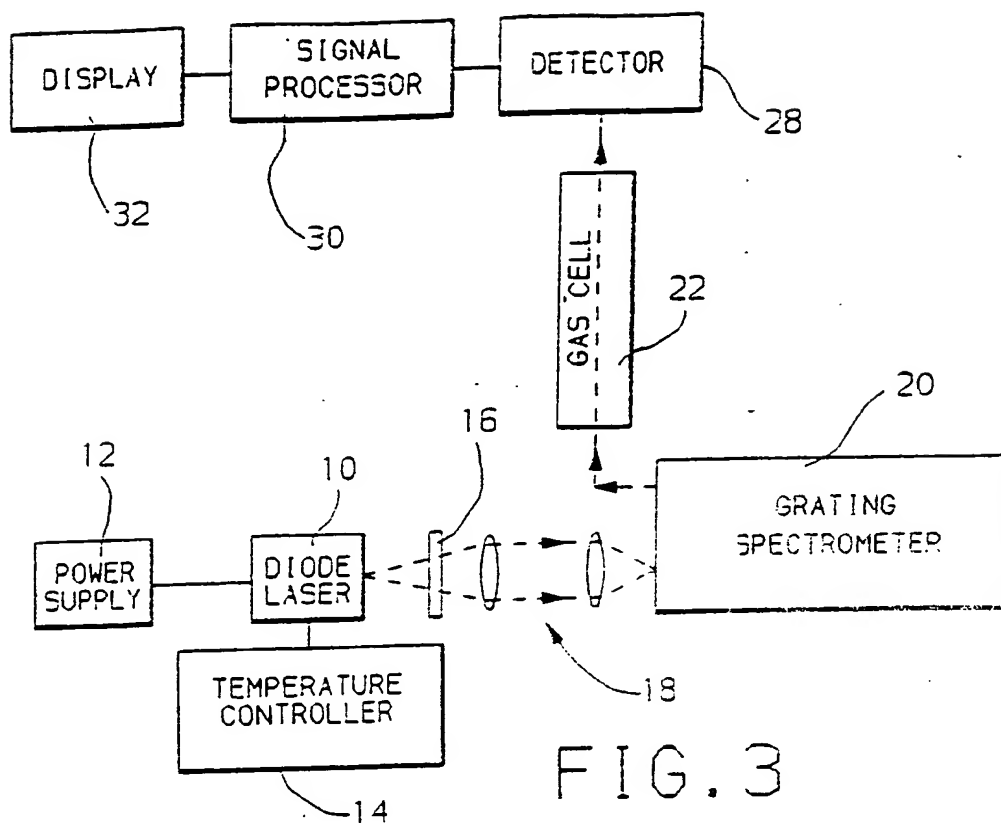


FIG. 3

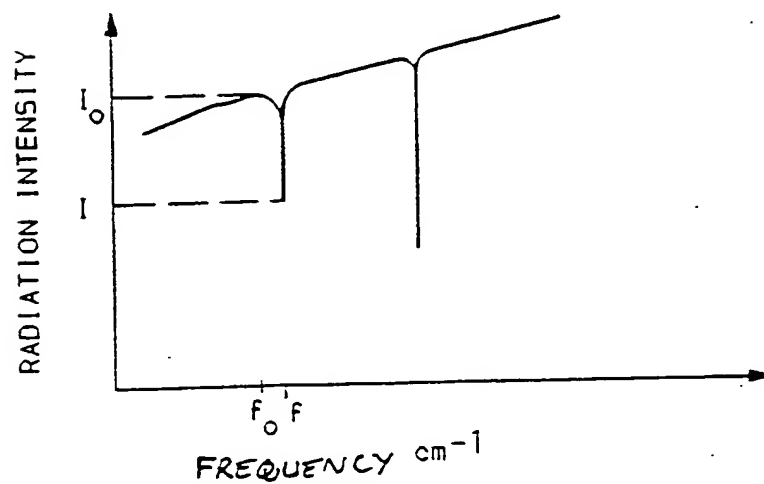


FIG. 4

PROCESS EVALUATION BY ISOTOPE ENRICHMENTField of the Invention

This invention relates to a stable isotope enrichment method for measuring the effects of a system or process on a substance and particularly to such a method using infra-red spectroscopy for direct measurement of species concentration.

Background of the Invention

Clinical biomedical testing as well as engineering or scientific testing often involves the evaluation of a process occurring in the human body or other system. One well-known test requires that a substance enriched with a tracer, usually in the form of an isotope, be measured to evaluate the effect of the system process on that substance.

The use of radio-isotopes for such tests is well documented and valuable information has been gathered in this manner. It is also well known that the radio-activity can have undesirable effects on human or other subjects exposed to the radiation and contamination of mechanical systems as well as waste disposal problems make the radio-active tracer techniques undesirable. Another drawback is that suitable radio-isotopes do not exist for every substance needed for testing.

Stable isotopes have also been used as tracers. The application of such tracers is discussed in the paper, "Stable Isotope Tracer in the Life Sciences and Medicine", Matwiyoff et al: Science, 181, 1125 (Sep. 1973). These stable tracers have the advantages of safety since exposure to radiation is not necessary. A further advantage is the wide variety of isotopic species available for use as tracers. The

abundant species naturally occurring in any system usually have chemically equivalent low abundance or rare isotopic species suitable for use in enrichment tests. Heretofore the practicality of such tests has
5 been limited by the lack of a good method of measuring the concentration of enriched species.

Mass spectroscopy is often used for such stable isotope measurements, particularly for
10 bio-medical applications, as set out in the above mentioned Science article. These systems can be made to be very accurate for ratio measurement but then for the most part, they are unable to make direct concentration measurements. One of the problems with
15 mass spectroscopy is that some substances such as carbon monoxide (CO) have mass redundancy, i.e., different isotopes of the same molecule can have the same nominal mass, so that mass separation cannot be used, thereby eliminating some very useful species as tracer substances. These mass spectrometers are very
20 expensive, highly trained operators are required and test results are delayed due to the extensive processing required for sample preparation. Because isotope ratio mass spectroscopy measures ratios of species of different mass, any interfering species
25 having the same nominal mass of the one to be measured are often present and must be removed prior to mass ratio measurement. This is not always feasible. The isotope ratio mass spectrometers are not general purpose in the sense of being applicable to a wide
30 variety of species. Rather, to afford sufficient sensitivity to resolve the species of similar mass, the instrument is dedicated to operation in a rather narrow

portion of the mass spectrum. As a result, the mass spectroscopy technique is useful as a research tool but is economically prohibited from becoming a widely available clinical instrument to meet the needs of the medical profession.

"Determination of Carbon-13 by Infrared Spectrophotometry of Carbon Monoxide", McDowell: Analytical Chemistry, 42, 1192 (1970) discloses that mass spectrometry might be avoided for the measurement of the $^{13}\text{CO}/^{12}\text{CO}$ ratio in a sample that appears to be pure CO. The relatively broad band of the spectrophotometer fails to resolve the individual isotopic lines but does accomplish a crude measure of the ratio. Neither an accurate ratio nor an absolute concentration can be determined by this technology. Another infra-red measurement has found utility in automotive emissions testing, but isotope enrichment is not used. As described in the paper by J. Hill et al: "Time-Resolved Measurement of Vehicle Sulfate and Methane Emissions with Tunable Diode Lasers", SAE 800510 (Feb. 1980), a tunable diode laser is used to scan absorption lines of molecules in exhaust gas samples without regard to isotopic species. The sample is at a pressure too high to allow the resolution of fine line structure representing individual isotopic lines so that only a gross measurement is made.

Infra-red absorption spectroscopy is known to be useful for the measurement of naturally occurring isotopic species. Sometimes the parameter being measured is the isotope spectrum, i.e., the wavelengths of the several absorption lines. This is shown by Jensen et al: Laser Focus, May 1976, which reveals

that tunable diode lasers were used to identify the spectra of naturally occurring uranium isotopes. The measurement of isotope concentrations is not taught. In other cases the measurement of the concentration of an isotope is important and measurement accuracy is stressed. For example, Labrie et al: Applied Physics, 24, 381 (1981), discloses a radio-carbon dating measurement using a tunable diode laser and a multi-pass optical cell for measuring carbon-14 concentration, although several hours are required for each measurement to achieve the accuracy called for by radio-carbon dating. The paper concludes that infra-red laser spectroscopy can be used for the measurement of small abundances of other stable and radioactive isotopes. While the technique is of interest, the long measurement time does not meet the needs of clinical testing. "Isotope Analysis by Infrared Laser Absorption Spectroscopy", Lehmann et al: Applied Physics 13, 153 (1977), discusses a tunable PBS laser to examine isotope-enriched carbon dioxide to identify the existence of absorption lines for each isotope and to measure absorption coefficients at different pressures. It is suggested that accurate results require a split-beam double-pass system.

The application of infra-red methods to isotope measurements for detecting stable isotopes in bio-medical applications is briefly considered in the above mentioned 1973 Science article by Matwiyoff et al. In that paper there is no description of a particular infra-red system. It discusses the technique of ingesting a ^{13}C -labelled substrate, breathing into an evacuated bulb, and determining the

excess ^{13}C in the breath carbon dioxide by an infra-red spectrometer. The difficulties in sample preparation are not revealed nor is the accuracy of the method. It is well known that the relatively broad band of any spectrometer available in that era does not very well
5 lend itself to separation of isotopic species. In another section the paper states that while infra-red methods require simple inexpensive instrumentation, they are limited mainly to simple gases and do not
10 provide information about the location of the isotope in the molecule and thus, by implication, are inferior to nuclear magnetic resonance (NMR) or mass spectrometry methods.

The most encouraging work indicating the practicality of infra-red spectroscopy used for stable
15 isotope measurements in biological or engineering systems is that of Lee et al as described in "Tunable Diode Laser Spectroscopy of Stable Isotopic Tracers-Detection and Measurement of Relative Abundance
20 of Isotopic Carbon Monoxide", Lee et al: Proceedings of the Second International Symposium on Synthesis and Applications of Isotopically Labeled Compounds, pages 441-446 (1986); "High Resolution Infrared Diode Laser Spectroscopy for Isotope Analysis - Measurement of
25 Isotopic Carbon Monoxide", Lee et al: Applied Physics Letters 48, 619 (Mar. 1986); U.S. Patent 4,684,805; and "The Clinical Spectrum", Scientific American, Dec, 1987. This work is referred to by Partin: Mat. Res.
Soc. Symp. Proc. Vol. 90, (1987). That work showed
30 that infra-red spectroscopy using a tunable diode laser and a dual path measurement cell in which one path is adjustable provides very accurate results and that it

is well suited to the measurement of ratios of isotopic species used in clinical tests of patients. The system is proposed as a simpler, far less expensive instrument than the mass spectrometer previously in use that could broaden the scope of tracer methodologies. That system uses a dual path sample cell in which one path is adjustable in order to gain simultaneous measurements of two isotopic species (normally comprising an abundant naturally occurring isotope and a much less abundant isotope which may be enriched) and the isotope ratio is measured with great accuracy. The present invention is a development of that work.

Summary of the Invention

It is therefore an object of the invention to provide a process evaluation method using infra-red spectroscopy to directly measure concentrations of isotopically-enriched materials.

It is another object to provide such a method capable of obtaining such measurements without resort to isotope ratio measurement.

It is a further object to provide such a method requiring simple single path apparatus, requiring minimal sample preparation and yielding rapid results.

In general, the invention is carried out by the method of evaluating a process in a system containing material amenable to isotope enrichment comprising the steps of treating the system with a substance enriched with a tracer isotope, after such treatment, preparing a gaseous sample of material from the system containing a tracer species enriched with the tracer isotope, transmitting monochromatic

radiation through the gaseous sample at the frequency of an absorption line for the enriched species, and detecting the intensity of a spectral line for the enriched species in the sample to determine the concentration or the enrichment value of the tracer species in the sample.

Brief Description of the Drawings

The above and other advantages of the invention will become more apparent from the following description taken in conjunction with the accompanying drawings, wherein like references refer to like parts and wherein:

Figures 1 and 2 are graphs with different vertical scales of spectral lines of carbon monoxide in human breath illustrating the technology utilized in the method of the invention,

Figure 3 is a schematic diagram of an apparatus for carrying out the method of the invention, and

Figure 4 is a graph of line intensities in a high resolution spectrum of isotopic species.

Description of the Preferred Embodiment

The method of the invention is described chiefly in terms of bio-medical applications, although some industrial process control or engineering testing applications will occur to those who become familiar with the invention. The success of the applications depends on making measurements with sufficient accuracy to suit the needs of the application with relatively inexpensive equipment. It has been demonstrated that a single path infra-red spectroscopic instrument with sensitive detection can produce signals with excellent

signal-to-noise ratio suitable for analysis of very low isotopic species analysis in human breath. Two spectroscopy techniques are presented here for use with the method of the invention, one technique being especially attractive for the accurate detection of low isotopic species concentration and the other technique being simpler and preferred for larger concentrations.

One of the techniques employs second harmonic detection. It is well known and need not be detailed here. Further information is found in the paper of Reid et al: "Second-Harmonic Detection with Tunable Diode Lasers - Comparison of Experiment and Theory", Appl. Phys. 326, 203-210 (1981) which is incorporated herein by reference. Figures 1 and 2, which differ only in that Figure 2 has an expanded vertical scale, show a second harmonic detection curve representing the absorption lines for natural isotopic abundance of carbon-12 and carbon-13 species of CO. The $^{13}\text{C}^{16}\text{O}$ peak represents a concentration of one to ten parts per hundred million. The lines were obtained for a sample of human breath from an individual with water vapor removed and no other processing. The sample was held at a pressure of 3200 Pa (24 torr), and was scanned through a path length of 20 metres by radiation from a tunable diode laser. The line peak is related to the concentration of the measured species by a working curve obtained by calibration of the instrument using standards of known concentration. This elegant procedure is exceptionally good for measurement of very low concentrations but a simpler more direct technique, described below, is used where larger concentrations are to be measured. In each case the

measurement of species ratios is not required. Each approach uses the same basic spectroscopy instrument.

Typical apparatus for carrying out the method is shown in Figure 3. The apparatus includes a tunable diode laser 10, a power supply 12 for the laser 10, and a temperature controller 14. The laser is conveniently of the lead salt type described in the U.S. Patents 4,350,990 and 4,186,355 to Lo and 4,577,322 and 4,608,694 to Partin, but may also be of the GaInAsSb types. Such lasers are tuned by varying the operating temperature and are available for operation in the wavelength range of 2.5 to 30 micrometres. The laser can be scanned over a small band, say about 0.5 to 1 cm^{-1} , at a rate of 500 cycles per second. The laser can also be tuned to emit at a pre-set wavelength without scanning action to specifically target an absorption peak, for example. Alternatively, scanning or sweeping action allows the entire absorption curve related to a single line to be measured in detail. By varying the injection current, operating parameters of the laser system can be adjusted for a variety of isotopes and molecules. Any infra-red active molecule with a suitable spectrum can be studied by this system. The system therefore would be versatile rather than dedicated to a single isotopic species. The isotopic spectral lines are well resolved, thus eliminating any background interference like that encountered in conventional mass spectrometry.

The GaInAsSb types of lasers mentioned above are in a class of shorter wavelength diode lasers composed of III-V compounds involving some of the following elements: Al, Ga, In, P, As and Sb, as

described by Caneau et al; "cw operation of GaInAsSb/AlGaAsSb lasers up to 190 K", Appl. Phys. Lett. 49, 55 (1986). These lasers may not emit at the fundamental vibration-rotation frequencies but are
5 utilized for combination or overtone bands along with the more sensitive detecting schemes for stable isotope analysis. These shorter wavelength lasers operate at relatively high heat-sink temperatures and with shorter wavelength infra-red detectors, thereby facilitating
10 the use of inexpensive coolers such as thermo-electric coolers, or require no cooling below room temperature.

Still another laser source is a band-aligned super-lattice laser which has the potential of being fabricated for room temperature operation. Such a
15 laser is described by Yuh et al; "Novel infrared band-aligned superlattice laser", Appl. Phys. Lett., 51, 1404 (1987).

Laser radiation passes through a chopper 16 and a lens system 18 to a grating spectrometer 20 which
20 passes a single optical mode. The laser is tuned so that this mode spans the absorption line of the desired isotopic molecule. The radiation then passes through a cell 22 containing the sample gas. A detector 28 senses the radiation which passes the cell 22 and a
25 signal processor 30 processes the detector signals and provides an output on display 32. In practicing the second harmonic detection technique the chopper can be omitted and the injection current is varied to modulate the radiation. In addition the signal processor is
30 equipped to analyze the signal in accordance with second harmonic detection. To carry out the method of the invention some variations on the basic apparatus

will occur to the user. For example, the small amount of noise present on the baseline of the Figure 2 curve is due mainly to optical noise originating in the refractive optics. That noise source can be
5 eliminated by the substitution of off-axis parabolic mirrors for the lenses. Then even smaller concentrations of an isotopic species can be measured without interference from noise. For single mode operation, the grating spectrometer can also be
10 by-passed.

Biological or engineering tests, for example, often involve the measurement of material which passes through a subject or system or undergoes a process which may involve a chemical change of the material.
15 According to the invention, by enriching the material with a stable isotope of a molecular constituent of the material, the amount of the material which passes to another part of the system or undergoes a chemical or biological change can be measured in terms of the
20 absolute concentration in a sample taken from the system or subject. The measurement is made on a single absorption line in the spectra of the tracer isotopic species. Such a line is selected from a region free from interference from other species and the sample
25 requires no preparation other than removal of water vapor and maintaining a low pressure to eliminate pressure broadening of the spectral lines. It is not necessary, as in the case of isotope ratio mass spectrometry to measure the ratio of the enriched
30 substance to another substance. Rather, the concentration is measured directly from the radiation intensity at the spectral line along with a measurement

of the incident radiation intensity. Possible errors in deriving concentration values from ratiometric techniques are avoided.

In bio-medical testing, a subject may be administered an isotopically-enriched or tracer substance and after the substance has undergone a physiological process it is deposited in a tissue or it is excreted. Very often such tests involve tracers which are converted to carbon dioxide, carbon monoxide, ammonia or water in the breath. Then a breath sample is taken by collecting a sample of breath that is exhaled from the subject. The water vapor is then removed from the sample (unless the water vapor itself is being tested) and the sample is introduced to a sample cell at a low pressure, say, of 3200 Da (24 torr). Radiation at the frequency of an absorption line of the isotopic tracer species of interest is transmitted through the cell and its intensity, I , is measured after passing through the cell. To determine the absolute concentration value a measure of the incident radiation intensity, I_0 , is needed. That value is obtained by evacuating the cell and measuring the intensity of the transmitted radiation at the same frequency after passing through the evacuated cell. As illustrated in Figure 4, another method of obtaining the incident radiation intensity, I_0 , is by tuning the frequency of the radiation to a value, f_0 , just off the line, that is, near the absorption line but not subject to absorption by that line. The concentration of the tracer species is determined from the Beer-Lambert law: $I = I_0 e^{-ap_l}$ where p is the partial pressure of the isotopic molecule (torr), l is the path length (cm)

and a is the spectral absorption coefficient of the isotopic molecule. In the case of breath samples, the isotopically-enriched substance is often enriched with carbon-13, oxygen-18 or nitrogen-15 and the resultant tracer species is usually carbon dioxide, carbon monoxide, water or ammonia. Thus two concentration measuring techniques are available without reference to other isotopic species. Each has its use depending on the physiological function under study.

As revealed in US patent 4,684,805, some studies using CO involve the intake of oxygen-18-labelled ozone ($^{18}\text{O}_3$) or nitrogen dioxide (N^{18}O_2) and determining the tracer deposited in tissues by preparing a gaseous sample containing CO from the tissue, and measuring the $\text{C}^{13}\text{O}/\text{C}^{16}\text{O}$ ratio and then determining the ratio enrichment in the sample. The present invention allows the direct measurement of the C^{18}O concentration without reference to a C^{16}O measurement. The gaseous sample is introduced to the sample cell at a low pressure. Then one of the concentration-measuring techniques is employed to obtain a direct measure of the tracer concentration in the sample.

The well-known glucose tolerance test is usually carried out by taking a series of blood samples following ingestion of a quantity of glucose, and then analyzing the blood samples to provide results at some later time. This invention allows an easier test procedure, especially from the viewpoint of the subject, and furnishes nearly immediate results. The glucose administered to the subject is labelled with carbon-13. The subject's breath is sampled initially

to obtain a reference sample and then sampled periodically following the oral administration of the labelled glucose. The labelled glucose is a precursor to carbon dioxide in the breath so that the samples are
5 measured to determine the carbon-13 isotopic species in the breath. Water is removed from each sample, usually by freezing or trapping, and the absolute concentration of the tagged CO_2 is measured in a few minutes. The concentrations of the samples reveal the profile of
10 the physiological processing of the glucose, and this is independent of the presence of CO_2 in the breath from any other source. The enrichment of each sample is determined by the simple comparison with the reference sample.

15 It will thus be seen that the method of the invention makes possible the investigation of a process by the use of stable isotopic tracers through the direct measurement of isotopic species concentration independently of other species in the sample. The
20 method takes advantage of known apparatus which is relatively inexpensive so that the clinical application of the method can be made available throughout the medical community, although industrial applications are also practical. The method requires minimal sample
25 preparation, especially when a gaseous sample is available, and the results can be obtained rapidly. In addition, for physiological applications the tests are, in most cases, conducted non-invasively.

Claims:

1. A method of evaluating a process in a system containing material amenable to isotope enrichment comprising the steps of: treating the system with a substance enriched with a tracer isotope; after such treatment, preparing a gaseous sample of material from the system containing a tracer species enriched with the tracer isotope; maintaining the gaseous sample at a pressure where a distinction between an absorption line of the tracer species and the absorption lines of related isotopic species is discernible; transmitting monochromatic radiation through the gaseous sample at the frequency of an absorption line for the enriched species; and detecting the intensity of a spectral line for the enriched species in the sample to determine the enrichment value of the tracer species in the sample.

2. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the method includes the steps of determining an incident radiation level by transmitting radiation through the sample at a radiation frequency just off the absorption line and detecting the incident intensity, and determining the enrichment value from the relative detected intensities.

3. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which a reference value of intensity is determined by detecting the radiation in the absence of an absorbing sample, and calculating the enrichment value from the ratio of the detected intensities.

4. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the transmitting step comprises modulating the radiation frequency and sweeping the radiation frequency over an absorption line of the enriched species, and the detecting step comprises harmonic detection of the transmitted radiation.

5. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the method includes the steps of: prior to the enrichment treatment, preparing a reference gaseous sample of material containing a non-enriched quantity of the tracer species; performing the transmitting and detecting steps to derive a reference spectral line intensity for the tracer species, and determining the enrichment value from the difference between the reference spectral line intensity and the enriched spectral line intensity.

6. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which a plurality of enriched samples are taken at different times or places and measured for enrichment values to determine variations in enrichment quantities.

7. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the method is that of evaluating a physiological function in a subject by a stable isotope tracer, and comprises the steps of: administering to the subject an

isotopically-enriched substance which is subject to the physiological function and which isotope is eventually expired in the form of an isotopically-enriched gaseous species; after such treatment, collecting a breath sample from the subject; maintaining the gaseous sample at a pressure sufficiently low as to allow a distinction to be made between an absorption line of the enriched species and the absorption lines of related isotopic species; transmitting said monochromatic radiation through the breath sample at the frequency of an absorption line of the enriched species, and detecting the intensity of the related spectral line for the enriched species as a measure of the concentration of the enriched species in the sample.

8. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the method is a method of evaluating a physiological function in a subject by a stable isotope tracer and comprises the steps of: administering to the subject an isotopically-enriched substance which is subject to the physiological function and which isotope is deposited as an enriched species in a biological specimen; after such treatment, preparing from the specimen a gaseous sample containing a specie enriched with the isotope tracer; maintaining the gaseous sample at a pressure sufficiently low to allow said distinction to be made between an absorption line of the enriched specie and the absorption lines of related isotopic species; transmitting said monochromatic radiation through the sample at the

frequency of an absorption line of the enriched species; and detecting the intensity of the related spectral line.

5 9. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 3, in which the stable isotope tracer is carbon-13 and the enriched species in the gaseous sample is carbon dioxide enriched with carbon-13.

10 10. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 3, in which the stable isotope tracer is a low abundance isotope of carbon or oxygen and the enriched species in the gaseous sample is carbon monoxide.

15 11. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 3, in which the stable isotope tracer is oxygen-18 and the enriched species in the gaseous sample is water vapor enriched with oxygen-18.

20 12. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 3, in which the stable isotope tracer is nitrogen-15 and the enriched species in the gaseous sample is ammonia enriched with nitrogen-15.

25 13. A method of evaluating a process in a system containing material amenable to isotope enrichment according to claim 1, in which the method is a method of evaluating a physiological process in a subject by infra-red spectroscopic analysis of an

30

- enriched species, and comprises the steps of:
- preparing a substance enriched with a tracer isotope which is a precursor of an enriched isotopic species to be measured; preparing a first gaseous sample from a
- 5 subject; transmitting infra-red radiation through the gaseous sample, at the frequency of an absorption line of the isotopic species to be measured; detecting the intensity of the resultant spectral line to measure the concentration of the isotopic species in the sample;
- 10 treating the subject with the isotope-enriched substance and then preparing a second gaseous sample from the subject; repeating the transmitting and detecting steps for the second sample; and comparing the measured concentrations of said isotope in said
- 15 first and second gaseous samples, to thereby reveal the effect of the physiological process on the enriched substance.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.